

**IN THE SPECIFICATION**

Please replace the paragraph bridging pages 7 and 8 with the following paragraph. The changes are shown explicitly in the attached "Version with Markings to Show Changes Made."

B The recombinase specific of said SSRTS is selected from the group of site—specific recombinases composed of the Cre recombinase of bacteriophage P1, the FLP recombinase of *Saccharomyces cerevisiae*, the R recombinase of *Zygosaccharomyces rouxii* pSR1, the A recombinase of *Kluyveromyces drosophilarius* pKD1, the A recombinase of *Kluyveromyces waltii* pKW1, the integrase  $\lambda$  Int, the recombinase of the GIN recombination system of the Mu phage, of the bacterial  $\beta$  recombinase or a variant thereof. In a preferred embodiment, the recombinase is the Cre recombinase of bacteriophage P1 (Abremski et. al., 1984), or its natural or synthetic variants. Cre is available commercially (Novagen, Catalog No. 69247—1). Recombination mediated by Cre is freely reversible. Cre works in simple buffers with either magnesium or spermidine as a cofactor, as is well known in the art. The DNA substrates can be either linear or supercoiled. A number of mutant LoxP sites have been described (Hoess et al., 1986; Lee et al., 1998), indeed, the corresponding SSRTS L1 and/or L2 specific for said Cre recombinase are chosen from the group composed of the sequences Lox P1 (ATCC 53 254 et 20 773), Lox 66, Lox 71, Lox 511, Lox 512, Lox 514, Lox B, Lox L, Lox R and mutated sequences of Lox P1 site harboring at least one point mutation in the 8 nucleotide spacer sequence. In one embodiment, the point mutation is substitution of A for G at position 7 of the eight base spacer sequence of the wild type Lox P1 sequence, referred to herein as Lox511 sequence. Preferred SSRTS are Lox P1 (SEQ ID NO. 1) and Lox 511 (SEQ ID NO. 53)

Please replace the paragraph bridging pages 26-27 with the following paragraph.

**Figure 2. Schematic representation of the construct pFIEXR and of the expected plasmids after Cre-mediated rearrangement.** (A) pFIEXR (SEQ ID NO. 54) contains, in the following order, the SV40 promoter (broken arrow), a loxP site (open arrowhead), a lox511 site (closed arrowhead), the coding sequence for the enhanced-green fluorescent protein (EGFP) linked to a poly-adenylation signal, the  $\beta$ -galactosidase promoter-less minigene (LacZ) in the antisense orientation, a loxP and a lox 511 sites in inverted orientations. The SV40 promoter first drives the expression of EGFP. (B) Intermediate step after Cre-mediated inversion at the loxP sites. (C) Intermediate step after Cre-mediated inversion at the lox 511 sites. (D) Final product after Cre-mediated excision between the two lox 511 or the two loxP sites (asterisks). In this plasmid, SV40 promoter now drives beta-galactosidase expression. This reaction is not reversible, as the final plasmid contains single loxP and lox511 sites, which cannot recombine together.

Please replace the paragraph bridging pages 27-28 with the following paragraph.

**Figure 3. *In vitro* Cre recombinase-mediated inversion/excision assay.** (A) Schematic drawing of the ploxLacZlox construct used to check for Cre preparation efficiency before (upper panel) and after (lower panel) Cre-mediated recombination. EcoRV restriction sites and location of probes 1 and 2 are indicated. (B) Schematic drawing of pFIEXR (SEQ ID NO. 54) before (upper panel) and after (lower panel, pFIEXRrec) Cre-mediated recombination. EcoRV and XbaI restriction sites, together with location of probes 1 and 2 are indicated. (C) Evidence for Cre-mediated recombination by Southern blot analysis of plasmids digested with EcoRV and XbaI using probe 1. Lane 1 and 2, loxP-flanked LacZ plasmid (ploxLacZlox) ; lane 3 and 4, pFIEXR; lane 5 and 6, pFIEXRrec (inverted/excised pFIEXR, see Materials and Methods). A crude Cre preparation was added in reactions illustrated in lanes 2, 4 and 6, whereas a heat-inactivated Cre preparation was added in reactions shown in lanes 1, 3 and 5. (D) Evidence for Cre-mediated recombination probing the same Southern blot as in (C) using probe 2 (for details see Materials and Methods). Note that the excised lacZlox fragment (3.7kb) , which does not contain plasmid sequences, was

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lost during amplification in bacteria. Open arrowhead, loxP site; closed arrowhead, lox511 site.

Please replace the paragraph bridging pages 28-29 with the following paragraph.

**Figure 5: Generation of a conditional RAR $\gamma$  allele by homologous recombination.**

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(A) Schematic drawing of the RAR $\gamma$  locus. Exons 7 to 14 are shown as solid boxes. As indicated, E7 is specific for RAR $\gamma$ 2, while E8 to E14 are common to all isoforms. The promoter (P2) is indicated by a broken arrow. 5' and 3' untranslated regions are shown as white boxes. Exon 8, whose splice acceptor is shown as waved lines, was chosen for the conditional disruption of RAR $\gamma$ . (B) Structure of the targeting vector (p $\gamma$ 6.0Flex $\beta$ -Gal) (SEQ ID NO. 55). (C) Structure of the recombinant allele following homologous recombination. (D) Structure of the recombinant allele after FLP-mediated removal of the selection cassette.

Please replace the paragraph bridging pages 31-32 with the following paragraph.

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**Figure 9: *In vitro* Cre recombinase-mediated inversion/excision assay on plasmid pJMG (SEQ ID NO. 56).** (A) Schematic drawing of pJMG (upper panel), the intermediate construct pJMG-f (middle panel) and the final construct pJMG-fx (lower panel). HindIII restriction sites, together with the location of the probe are indicated. (B) Evidence for Cre-mediated recombination assessed by ethidium bromide stained agarose gel analysis of HindIII digested plasmids. Lane 1 and 2, loxP-flanked LacZ plasmid (ploxLacZlox); lane 3 and 4, pJMG; lane 5 and 6, pJMG-f (inverted pJMG, see Materials and Methods); lane 7 and 8, pJMG-fx (inverted and excised pJMG, see Materials and Methods). A Cre preparation was added in the reactions illustrated in lanes 2, 4, 6 and 8, whereas a heat-inactivated Cre preparation was added in the reactions shown in lanes 1, 3, 5 and 7. The sizes of the expected HindIII fragments are indicated on the right. (C) Evidence for Cre-mediated recombination assessed by Southern blot using a probe recognizing the rabbit beta-globin splice acceptor site (for details see Materials and Methods). Note that this probe does not hybridise to the

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Cont ploxLacZlox. Open arrowhead, loxP site; closed arrowhead, lox511 site; closed flag, FRT site; open flag, FRTm site, SD, synthetic splice donor.

Please replace the paragraph bridging pages 35-36 with the following paragraph.

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1.1. **DNA Constructs.** To construct plasmid pFIEXP (SEQ ID NO. 54) (Fig. 1A), a loxP site, in the sense orientation, followed by a 21-bp spacer (oligos R1/R2; Table 1) was introduced into the EcoRI site of pSG5 (Green *et al.*, 1988). A lox511 site (Hoess *et al.*, 1986), also in the sense orientation, followed by a 21-bp spacer (oligos R3/R4) was introduced 3' to the loxP site. A second loxP site, in the antisense orientation, followed by a 21-bp spacer (oligos R5/R6) was introduced 3' to the first loxP and lox511 sites. A second lox511 site, also in the antisense orientation, followed by a 21-bp spacer (oligos R7/R8), was introduced 3' to the latter loxP site. The coding sequence of the enhanced green fluorescent protein (Zhang *et al.*, 1996) (EGFP; PCR-amplified using oligos R9/R10) and an NLS- $\beta$ -galactosidase pA cassette (LacZ) (Bonnerot *et al.*, 1987) were introduced between the two sets of loxP sites, in the sense and the antisense orientation, respectively. Finally, the remaining LacZ sequences of pSG5 were removed by digestion with BsaAI and SfiI, and repair by homologous recombination in *E. coli* using a SV40 promoter fragment (PCR amplified using oligos R11/R12). All cloning steps were checked by sequencing. The final constructs were again sequenced in all modified parts before starting *in vitro* Cre-mediated recombination or cell culture experiments. Modifications were all carried out following standard protocols (Ausubel *et al.*, 1989). To obtain plasmid pFIEXRrec, pFIEXR was incubated with the Cre preparation (see below), and the recombined DNA was cloned in *E. coli*. pFIEXRrec structure was checked by restriction mapping and sequencing of the regions containing loxP and lox511 sites. Plasmids ploxlacZlox and pSG5-Cre have been described elsewhere (Feil *et al.*, 1997).

Please replace the table title and table on page 37 with the following table title and table. (Underlining in original.)

**Table 1. Sequences of primers used for construction of the pFLEXR plasmid (SEQ ID NO. 54).**

Name	Sequence
R1	5' – ATTGATAACTTCGTATAGCATACATTATACGAAGTTATCCAAGCTTCAC CATCGACCCG-3' (SEQ ID NO. 1)
R2	5' – AATTCGGGTCGATGGTGAAGCTTGGATAACTTCGTATAATGTATGCTAT ACGAAGTTATC-3' (SEQ ID NO. 2)
B7 R3	5'- AATTGCCAAGCATCACCATCGACCCATAACTTCGTATAGTATACATTAT ACGAAGTTATCG-3' (SEQ ID NO. 3)
R4	5'- AATTCGATAACTTCGTATAATGTATACTATACGAAGTTATGGGTCGATG GTGATGCTTGGC-3' (SEQ ID NO. 4)
R5	5'- CTAGTGGATCCGATAACTTCGTATAATGTATGCTATACGAAGTTATCCA AGCATCACCATCGACCCT-3' (SEQ ID NO. 5)
R6	5'- CTAGAGGGTCGATGGTGTGCTTGGATAACTTCGTATAGCATACATTAT ACGAAGTTATCGGATCCA-3' (SEQ ID NO. 6)
R7	5'- CTAGTCCAGATCTCACCATCGACCCATAACTTCGTATAATGTATACTAT ACGAAGTTATT-3' (SEQ ID NO. 7)
R8	5'- CTAGAATAACTTCGTATAGTATACATTATACGAAGTTATGGGTCGATGG TGAGATCTGGA-3' (SEQ ID NO. 8)

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- R9 5'-GGGGAATTCTTCTTGTACAGCTCGTCCA-3' (SEQ ID NO. 9)  
R10 5'-GGGGAATTCCCATGGTGAGCAAGGGCGAGGAG-3' (SEQ ID NO. 10)  
R11 5'-CTATCAGGGCGATGGCCCACTACGTGTTCTGAGGCGGAAAGAACCA-3'  
(SEQ ID NO. 11)  
R12 5'-GGAATAGCTCAGAGGCCGAGGCGGCCTCGGCCTCTGCATAAATAAAA-  
3' (SEQ ID NO. 12)
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Please replace the second paragraph on page 38 with the following paragraph.

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**1.2. *In vitro* Cre reactions.** To perform Cre-mediated rearrangements *in vitro*, bacterial extracts containing an active Cre were prepared from *E. coli* 294-Cre strain 43. Cells were grown overnight at 37°C in 500ml LB medium, harvested by centrifugation, resuspended in 10ml Cre Buffer (50mM Tris/HCl pH 7.5, 33mM NaCl, 10mM MgCl<sub>2</sub>, 5% glycerol, 0.02% NaN<sub>3</sub>), and lysed by sonification. The soluble supernatant containing the Cre recombinase (Cre preparation) was recovered by centrifugation (14000 x g, 15min, 4°C). The relevant plasmids (3μg) were incubated with 100μl of the Cre preparation for 1 hour at 37°C. For the control reactions, Cre was heat-inactivated by incubating the Cre preparation for 10 min at 70°C. Plasmids were then isolated using the standard alkaline lysis method for DNA preparation (Ausubel *et al.*, 1989). The recovered DNA was then used to transform competent XL1-Blue cells, which were grown overnight in 2ml of LB at 37°C. Plasmids were isolated, digested by EcoRV and XbaI, separated on agarose gels and analyzed by Southern blotting using the radio-labelled oligos 5'-GTGCATCTGCCAGTTTGAGG-3' (SEQ ID NO. 13) or 5'-AATACGACTCACTATAG-3' (SEQ ID NO. 14) recognizing lacZ sequence or T7 promoter, respectively.

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Please replace the paragraph bridging pages 39-40 with the following paragraph.

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**1.4. Construction of plasmid pγ6.0FlExβ-Gal (SEQ ID NO. 55).** To construct plasmid pγ6.0FlExβ-Gal, the RARγ exon 8 splice acceptor (oligos G3/G4; Table 2) was

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inserted by homologous recombination in *E. coli* into an XbaI digested pBluescript SK+ (Pharmacia) containing a loxP site (oligos G1/G2) in the sense orientation at its NotI site and from which the LacZ sequences were removed. After insertion of a 62 bp fragment (oligos G5/G6) into the XbaI site, the (NLS)  $\beta$ -gal pA cassette (Bonnerot *et al.*, 1987) was introduced by homologous recombination in *E. coli*. A SnaBI and a lox511 site (oligos G7/G8) in the sense orientation was then introduced 5' of the loxP site into the SacII site. A second SnaBI site (oligo G9) was inserted into the BamHI site. An FRT site (oligos G10/G11) was inserted into the NotI site. The FRT/PGK/Neo/pA/FRT cassette was inserted into the XbaI site oligos G10/G11 giving rise to plasmid ploxP/lox511/lacZ/Neo. A loxP site was inserted in the sense orientation into the HpaI site of pSK $\gamma$ 6.0 (Lohnes *et al.*, 1993) (oligos G12/G13). A lox511 site was introduced in the sense orientation into the 3' reconstructed HpaI site (oligos G14/G15). The EcoRI insert of this plasmid was ligated into a pGEX4T3 to obtain a LacZ sequence-deficient vector (pGEX $\gamma$ 6.0-loxP-lox511). The SnaBI fragment from plasmid plox P-lox511-lac2-Neo was isolated and inserted into the SfiI site of pGEX $\gamma$ 6.0/loxP/lox511 to obtain py6.0Flex $\beta$ -Gal (SEQ ID NO. 55).

Please replace the table bridging pages 40-41 with the following table. (Underlining in original.)

**Table 2: Sequences of primers used for construction of the py6.0F1Ex $\beta$ -Gal plasmid (SEQ ID NO. 55).**

Name	Sequences
G1	5'-GGCCGCATAACTTCGTATAATGTATGCTATACGAAGTTAT- 3' (SEQ ID NO. 15)
G2	5'-GGCCATAACTTCGTATGCATACATTATACGAAGTTATGC-3' (SEQ ID NO. 16)
G3	5'-TATAATGTATGCTATACGAAGTTATTCCTTGGCCTGGAATTTCAGATT-3' (SEQ ID NO. 17)

- G4 5'-GCCCCGGGGGATCCACTAGTTCTAGATGTCTCCACCGCTGAATGAAAA  
GCA-3' (SEQ ID NO. 18)
- G5 5'-CTAGTATGGATAAAGTTTTCCGGAATTCCGCTCTAGACTCATCAATGT  
TATCTTATCATGTCTA-3' (SEQ ID NO. 19)
- G6 5'-CTAGTAGACATGATAAGATAACATTGATGAGTCTAGAGCGGAATTCCG  
GAAACTTTATCCATA-3' (SEQ ID NO. 20)
- G7 5'-GCTACGTAATAACTTCGTATAATGTATACTATACGAAGTTATGGGTCG  
ATGGTGAGATCTCCGC-3' (SEQ ID NO. 21)
- G8 5'-GGAGATCTCACCATCGACCCATAACTTCGTATAGTATACATTATACGA  
AGTTATTACGTAGCGC-3' (SEQ ID NO. 22)
- G9 5'-GATCTTACGTAA-3' (SEQ ID NO. 23)
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cont G10 3'-GGCCGGGAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCCC-3'  
(SEQ ID NO. 24)
- G11 5'-GGCCGGGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCCC-3'  
(SEQ ID NO. 25)
- G12 5'-AAGATAACTTCGTATAATGTATGCTATACGAAGTTATCCAAGCATCAC  
CATCGACCCGTT-3' (SEQ ID NO. 26)
- G13 5'-AACGGGTCGATGGTGATGCTTGGATAACTTCGTATAGCATACATTATA  
CGAAGTTATCTT-3' (SEQ ID NO. 27)
- G14 5'-AAGCCAAGCATCACCATCGACCCATAACTTCGTATAATGTATACT  
ATACGAAGTTATGTT-3' (SEQ ID NO. 28)



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G15 5'-AACATAACTTCGTATAGTATACATTATACGAAGTTATGGGTCGATGGT  
GATGCTTGGCTT-3' (SEQ ID NO. 29)

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Please replace the paragraph bridging pages 41-42 with the following paragraph.

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**1.5. Generation of the gene trap construct**

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To construct the plasmid pJMG (SEQ ID NO. 56), a PCR amplified PGK Neo cassette containing the OBS sequence and the synthetic splice donor site (SD; oligos J1/J2; Table 3) was introduced into the EcoRI site of pBluescript SK+ resulting in pJMG1. A cassette containing the FRT, loxP and lox511 sites was prepared by subsequent insertion of oligos J3 to J8 into a shuttle vector. This cassette was recovered by NruI and HindIII digest, repaired and introduced in front of the PGK-Neo gene of pJMG1. The lacZ sequence of the pBluescript SK+ was removed from pJMG1. A lox511 site (oligos J9/J10) and a FRTm site (oligos J11/J12) were subsequently introduced 3' to the synthetic splice donor site. The  $\beta$ -globin splice acceptor site (SA) followed by the IRES sequence were amplified by overlap extension PCR using oligos J13-J16. This fragment was introduced between the loxP site and the nls-LacZ polyA minigene of plasmid ploxP-nls-LacZ-pA. The obtained loxP-SD-IRES-nls-LacZ-pA DNA fragment was recovered and introduced, in antisense orientation at the BamHI site located in between the lox511 site and the PGK promoter to give to pJMG. The gene trap construct was excised from pJMG by NotI digestion and purification on a sucrose gradient.

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Please replace the table bridging pages 43-44 with the following paragraph.  
(Underlining in original.)

**Table 3: Sequences of primers used for construction of the plasmid pJMG.**

Name	Sequences
J1	5'- ACTAGTGGATCCCCCGGGCTGCAGGAATTCTACCGGGTAGGGGAGGCGC TT-3' (SEQ ID NO. 30)
J2	5'-GTATCGATAAGCTTGATATCGCCGCTCGAGACTTACCTGACTGGCCGTC GTTTACAGTCAGAAGAACTCGTCAAGAAG-3' (SEQ ID NO. 31)
J3	5'-CTCGCGAGGAATTCAACCAGAAGTTCCTATTCTCTAGAAAGTATAGGAA CTTCCAGCT-3' (SEQ ID NO. 32)
J4	5'-GGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCTGGTTGAATTCCTC GCGAGAGCT-3' (SEQ ID NO. 33)
J5	5'-AATGCCTACCGGACCATCATAACTTCGTATAATGTATACTATACGAAGT TATAAGCTTGCA-3' (SEQ ID NO. 34)
J6	5'-AGCTTATAACTTCGTATAGTATACATTATACGAAGTTATGATGGTCCGG TAGGCATTTGCA-3' (SEQ ID NO. 35)
J7	5'-GAGCTCATAACTTCGTATAATGTATGCTATACGAAGTTATCCAAGCATC ACCATATGCA-3' (SEQ ID NO. 36)
J8	5'-TATGGTGATGCTTGGATAACTTCGTATAGCATAATTATACGAAGTTAT GAGCTCTGCA-3' (SEQ ID NO. 37)
J9	5'-TCGACATAACTTCGTATAATGTATACTATACGAAGTTATAC-3' (SEQ ID NO. 38)
J10	5'-TCGAGTATAACTTCGTATAGTATACATTATACGAAGTTATG-3' (SEQ ID NO. 39)
J11	5'-TCGAAGAAGTTCCTAATCTATTTGAAGTATAGGAACTTCGCGGCCGCA- 3' (SEQ ID NO. 40)

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cont
- J12 5'-TCGATGCGGCCGCGAAGTTCCTATACTTCAAATAGATTAGGAACTTCT-3' (SEQ ID NO. 41)
- J13 5'-CCGGTCCTTGGCCTGGAATTTGCACTCTGTTGACAACCATTGTCTCCT-3' (SEQ ID NO. 42)
- J14 5'-GTAATACGACTCACTATAGGGAATTCCGCCCCCTCTCCCTC-3' (SEQ ID NO. 43)
- J15 5'-GAGGGAGAGGGGCGGAATTCCCTATAGTGAGTCGTATTAC-3' (SEQ ID NO. 44)
- J16 5'-CTCCACCGCTGAATGAAAAGCAGCATGGTTGTGGCAAGCTTATCAT-3' (SEQ ID NO. 45)

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Please replace the paragraph on page 44 with the following paragraph.

#### 1.6. *In vitro* Cre reaction for poly A trap experiments

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To test for functionality of loxP and lox511 sites of pJMG, an *in vitro* Cre reaction was carried out (Schnütgen *et al.*, 2001). Briefly, a crude extract of *E. coli* 294-Cre cells (Cre preparation; Buchholz *et al.*, 1996) was incubated with 3µg of the plasmids and the resulting DNA was transformed into *E. coli* DH5α and directly amplified in liquid medium. Amplified plasmid DNA was recovered and analysed by Southern blotting using the probe 5'-TAACAATTTACACAGGA-3' (SEQ ID NO. 46), recognising the rabbit β-globin intron splice acceptor sequence (Green *et al.*, 1988), to reveal the Cre-mediated rearranged constructs. To obtain the pJMG-f plasmid (Fig. 8B) pJMG was incubated for 5 min with the Cre preparation and transformed into *E. coli* DH5α. Individual clones were picked and analysed by restriction mapping and sequencing.

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Please replace the paragraph on page 45 with the following paragraph.

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### 1.8 RACE PCR

B14 3' RACE was carried out as described by Frohman (1994). Briefly, a first RT-PCR was carried out using the oligo nucleotides Qt (5' -CCAGTGAGCAGAGTGACG AGGACTCGAGCTCAAGCT17-3') (SE ID NO. 47) as anchor primer, as well as Q0 (5' -CCAGTGAGCAGAGTGACG-3') (SEQ ID NO. 48) and Neol (3' -ACCGCTTCCTCGTGGTTTAC-3') (SEQ ID NO. 49) for amplification. An aliquot of 1 µl of this reaction was used for a nested amplification using Q1 (5' -GAGGACTCGAGCTCAAGC-3') (SEQ ID NO. 50) and Neo2 (5' -GCCTTCTTGACGAGTTCTTC-3') (SEQ ID NO. 51) primers. The resulting PCR fragments were purified using the NucleoSpin kit (Macherey-Nagel) and sequenced using the Neo2 or OBS (5' -CTGTAAAACGACGGCCAGTC-3') (SEQ ID NO. 52) primers.

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Please replace the paragraph bridging pages 45-47 with the following paragraph.

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#### **EXAMPLE 1: *In vitro* site-specific recombination**

B15 The principle of the inventors' novel recombination strategy is illustrated in Figure 1. pFlExR (SEQ ID NO. 54), a pSG5-based reporter plasmid was designed (Figure 2) to test its feasibility. It contains one pair of wild type loxP sites (open arrowheads), and one pair of lox511 sites (closed arrowheads), the loxP sites within each pair being oriented head to head. This organization (i.e. alternate loxP, lox511 and again loxP, lox511) is important. Both loxP and lox511 sites are recognized by Cre recombinase; however, they are "incompatible", as lox511 sites can efficiently recombine with themselves, but not with loxP sites (Hoess *et al.*, 1986). Between the two sets of loxP-lox511 sites, the plasmid contains the coding region for the enhanced green-fluorescent protein (EGFP) in the sense orientation, and a promoter-less LacZ reporter gene in the antisense orientation. In this reporter plasmid, the SV40 promoter first directs expression of EGFP (Fig. 2A). Cre-mediated recombination may initially induce inversion of the intervening DNA at either the loxP sites (Fig. 23, open arrowheads), or the lox511 sites (Fig. 2B, closed arrowheads). Due to the reversibility of these reactions, an equilibrium between the states (A) and (B or C) is formed. However, inversion induces a

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direct repeat of either two lox511 sites (Fig. 2B; closed arrowheads) or two loxP sites (Fig. 2C; open arrowheads). A further Cre-mediated excision will then remove the DNA located between the two loxP or between the two lox511 sites (Fig. 2B and C; asterisks). In the resulting plasmid (pFlExRrec), single loxP and lox511 sites are left, making further inversion of the intervening DNA impossible (Fig. 2D). The SV40 promoter now drives expression of LacZ, instead of EGFP.

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